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Genome-wide characterization of N-terminal acetylation sites in *Saccharomyces cerevisiae*. Post-translational modifications of proteins play critical roles in the regulation of fundamental cellular activities. In the budding yeast *Saccharomyces cerevisiae*, N-terminal acetylation of proteins has been increasingly studied in recent years. Up to now, there has been no genome-wide profiling of N-terminal acetylation sites. In this work, we used capillary electrophoresis-laser-induced fluorescence (CE-LIF) to analyze acetylation sites in *S. cerevisiae*. We developed a new approach to acetylated peptides, by which the standard mixture was first digested and then ionized in the mass spectrometer. After the N-terminal acetylation sites were identified from the MS data, we performed in-depth analysis on the catalytic mechanism of the N-terminal acetylation sites and determined the genome-wide profiles of N-terminal acetylated proteins. More than 700 acetylation sites were detected, and more than 200 proteins were found to be N-terminally acetylated. In conclusion, we provide a comprehensive genomic landscape of N-terminal acetylated proteins in *S. cerevisiae*. Moreover, our strategy can be readily applied to characterize N-

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